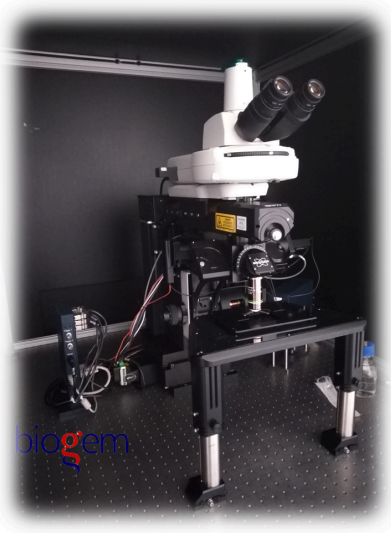

Multiphoton microscopy is the gold standard approach to study the functions and morphologies of organs in live animals at the cellular and subcellular level. In contrast to histological microscopy techniques, the intravital approach allows the simultaneous acquisition of morphological and functional informations: these informations can be acquired continuously in real time, whereas alternative techniques usually provide a snap shot of certain events. Compared to other microscopy approaches, such as confocal microscopy, the imaging resolution of multiphoton microscopy is higher, the penetration into the tissue is deeper, and the degree of phototoxicity is limited. Consequently, intravital multiphoton microscopy has been used to study the physiology and pathophysiology of various organs, such as skin, respiratory system, reproductive system, liver, gastrointestinal tract, pancreas, kidney, spleen, central nervous system, and heart. Furthermore, intravital multiphoton microscopy is a useful tool for immunology and cancer research. Multiphoton microscopy can be applied to both *in vivo* and *in vitro* preparations, tissue slices, and isolated perfused organs. It is suitable to carry out drug toxicity studies, such as the effects of some antibiotics used to treat bacterial infections. Studies of animals injected with nuclear dyes, can be advantageous to assess acute effects of insults on cell necrosis and apoptosis. Fluorescent dyes are widely used to visualize different cell types and compartments in vivo. Up to 4 different dyes can be concurrently excited by 2-photon absorption, allowing multiple comparation of labelled probes and simultaneous characterization of different parameters. Because vasculature dyes are predominantly retained by the vasculature, they can also be used to address abnormalities in vascular permeability. Alternatively, label-free imaging techniques such as second harmonic generation can be used to visualize intrinsic endogenous molecules, collagen deposition, the reduced form of nicotinamide adenine dinucleotide (NADH) and microtubules.


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